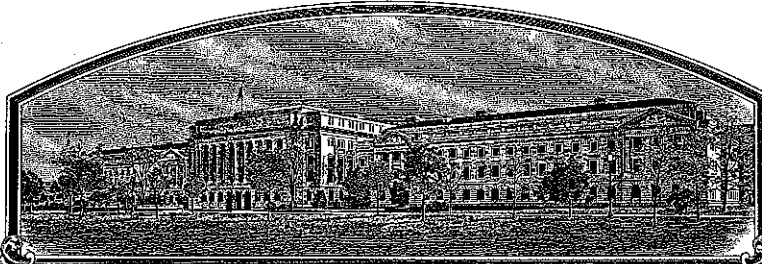


No.

200500311



# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Washington State University Research Foundation

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSES, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

WHEAT, COMMON

'Louise'

In Testimony Whereof, I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affixed at the City of Washington, D.C. this fifth day of June, in the year two thousand and six.

Attest:

Commissioner  
Plant Variety Protection Office  
Agricultural Marketing Service

Secretary of Agriculture



U.S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL MARKETING SERVICE  
SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICE

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE  
(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF OWNER <b>Washington State University Research Foundation</b>		2. TEMPORARY DESIGNATION OR EXPERIMENTAL NAME <b>WA007921</b>	3. VARIETY NAME <b>Louise</b>
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code, and Country) <b>1610 NE Eastgate Blvd. Pullman, WA 99163</b>		5. TELEPHONE (include area code) <b>(509) 335-4363</b>	<b>FOR OFFICIAL USE ONLY</b> PRO NUMBER <b>20050031</b> FILING DATE <b>August 4, 2005</b> FILING AND EXAMINATION FEES: \$ <b>3652.00</b> DATE <b>8/04/2005</b> CERTIFICATION FEE: \$ <b>768.00</b> DATE <b>April 7, 2006</b>
7. IF THE OWNER NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION (corporation, partnership, association, etc.) <b>Corporation</b>		6. FAX (include area code) <b>(509) 335-7237</b>	
8. IF INCORPORATED, GIVE STATE OF INCORPORATION <b>WA</b>	9. DATE OF INCORPORATION <b>July 7, 1939</b>		
10. NAME AND ADDRESS OF OWNER REPRESENTATIVE(S) TO SERVE IN THIS APPLICATION. (First person listed will receive all papers) <b>Dr. Keith Jones, Director Washington State University Research Foundation 1610 NE Eastgate Blvd. Pullman, WA 99163</b>			
11. TELEPHONE (Include area code) <b>(509) 335-4363</b>	12. FAX (Include area code) <b>(509) 335-7237</b>	13. E-MAIL <b>jonesk@wsu.edu</b>	
14. CROP KIND (Common Name) <b>Spring wheat</b>	16. FAMILY NAME (Botanical) <b>Gramineae</b>	18. DOES THE VARIETY CONTAIN ANY TRANSGENES? (OPTIONAL) <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO IF SO, PLEASE GIVE THE ASSIGNED USDA-APHIS REFERENCE NUMBER FOR THE APPROVED PETITION TO DEREGULATE THE GENETICALLY MODIFIED PLANT FOR COMMERCIALIZATION.	
15. GENUS AND SPECIES NAME OF CROP <b>Triticum aestivum L.</b>	17. IS THE VARIETY A FIRST GENERATION HYBRID? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO	20. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD AS A CLASS OF CERTIFIED SEED? (See Section 83(a) of the Plant Variety Protection Act) <input type="checkbox"/> YES (If "yes", answer items 21 and 22 below) <input checked="" type="checkbox"/> NO (If "no", go to item 23)	
19. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED (Follow instructions on reverse)		21. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES? <input type="checkbox"/> YES <input type="checkbox"/> NO IF YES, WHICH CLASSES? <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED	
a. <input checked="" type="checkbox"/> Exhibit A. Origin and Breeding History of the Variety b. <input checked="" type="checkbox"/> Exhibit B. Statement of Distinctness c. <input checked="" type="checkbox"/> Exhibit C. Objective Description of Variety d. <input checked="" type="checkbox"/> Exhibit D. Additional Description of the Variety (Optional) e. <input checked="" type="checkbox"/> Exhibit E. Statement of the Basis of the Owner's Ownership f. <input checked="" type="checkbox"/> Voucher Sample (2,500 viable untreated seeds or, for tuber propagated varieties, verification that tissue culture will be deposited and maintained in an approved public repository) g. <input checked="" type="checkbox"/> Filing and Examination Fee (\$3,652), made payable to "Treasurer of the United States" (Mail to the Plant Variety Protection Office)		22. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS? <input type="checkbox"/> YES <input type="checkbox"/> NO IF YES, SPECIFY THE NUMBER 1,2,3, etc. FOR EACH CLASS. <input type="checkbox"/> FOUNDATION <input type="checkbox"/> REGISTERED <input type="checkbox"/> CERTIFIED (If additional explanation is necessary, please use the space indicated on the reverse.)	
23. HAS THE VARIETY (INCLUDING ANY HARVESTED MATERIAL) OR A HYBRID PRODUCED FROM THIS VARIETY BEEN SOLD, DISPOSED OF, TRANSFERRED, OR USED IN THE U.S. OR OTHER COUNTRIES? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. (Please use space indicated on reverse.)		24. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT (PLANT BREEDER'S RIGHT OR PATENT)? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO IF YES, PLEASE GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. (Please use space indicated on reverse.)	

25. The owners declare that a viable sample of basic seed of the variety has been furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate.

The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Owner(s) is (are) informed that false representation herein can jeopardize protection and result in penalties.

SIGNATURE OF OWNER <b>Keith Jones</b>		SIGNATURE OF OWNER	
NAME (Please print or type) <b>Dr. Keith Jones</b>		NAME (Please print or type)	
CAPACITY OR TITLE <b>Director</b>	DATE <b>8/2/05</b>	CAPACITY OR TITLE <b>Director</b>	DATE

**GENERAL:** To be effectively filed with the Plant Variety Protection Office (PVPO), **ALL** of the following items must be **received** in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to **reproduce** the variety, or for tuber reproduced varieties verification that a viable (*in the sense that it will reproduce an entire plant*) tissue culture will be deposited and maintained in an approved public repository; (4) check drawn on a U.S. bank for \$3,652 (\$432 filing fee and \$3,220 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice.) Partial applications will be held in the PVPO for not more than 90 days, then returned to the applicant as unfilled. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 401, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. **DO NOT** use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amount of \$432 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

**Plant Variety Protection Office**

**Telephone: (301) 504-5518**

**FAX: (301) 504-5291**

**Homepage:** <http://www.ams.usda.gov/science/pvpo/pvpindex.htm>

To avoid conflict with other variety names in use, the applicant must check the appropriate recognized authority and provide evidence that name has been cleared by the appropriate recognized authority before the Certificate of Protection is issued. For example, for agricultural and vegetable crops, contact: Seed Branch, AMS, USDA, 10301 Baltimore Avenue, Suite 401 NAL Building, Beltsville, MD 20705. Telephone: (301) 504-5682 <http://www.ams.usda.gov/lsg/seed.htm>.

## ITEM

- 19a. Give: (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;  
(2) the details of subsequent stages of selection and multiplication;  
(3) evidence of uniformity and stability; and  
(4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified
- 19b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:  
(1) identify these varieties and state all differences objectively;  
(2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and  
(3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 19c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 19d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, etc.
- 19e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
20. If "Yes" is specified (*seed of this variety be sold by variety name only, as a class of certified seed*), the applicant **MAY NOT** reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. (See *Regulations and Rules of Practice*, Section 97.103).
23. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
24. See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.

## 22. CONTINUED FROM FRONT (Please provide a statement as to the limitation and sequence of generations that may be certified.)

NA

## 23. CONTINUED FROM FRONT (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)

Sold as Foundation seed on 2/24/05.

## 24. CONTINUED FROM FRONT (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)

NA

**NOTES:** It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. The fees for filing a change of address; owner's representative; ownership or assignment; or any modification of owner's name is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, sexual orientation, marital or family status, political beliefs, parental status, or protected genetic information. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at 202-720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

**EXHIBIT A - BREEDING HISTORY****'Louise'**

**1. Genealogy:** 'Wakanz' (PI 506352)/'Wawawai' (PI 574538)

**2. Stages of Selection and Multiplication:**

**1992:** Final cross made: WSU research land.

**1993:** F<sub>1</sub> generation; advanced on WSU research land; all plants uniform; no variants observed.

**1994:** F<sub>2</sub> bulk population; WSU research land; selected 100 random spikes; segregating for maturity, plant height, and disease resistance; no variants observed.

**1995:** F<sub>3</sub> bulk population; WSU research land; no selection applied; segregating for maturity, plant height, and disease resistance; no variants observed.

**1996:** F<sub>4</sub> bulk population; WSU research land; selected 150 random spikes; segregating for maturity, plant height, and disease resistance; no variants observed.

**1997:** F<sub>4:5</sub> head row; WSU research land; selected based on appropriate plant height, head type, maturity, and disease resistance; no variants were observed within the single row.

**1998:** F<sub>4:6</sub> Single Plot Nursery (tested as S9800189); WSU research land; selected based on appropriate plant height, head type, maturity, field resistance to stripe rust, grain protein content, test weight, grain yield, and milling/baking quality; no variants were observed within the plot.

**1999:** F<sub>4:7</sub> Preliminary Yield Trial; WSU research land; selected based on appropriate plant height, head type, maturity, field resistance to stripe rust, grain protein content, test weight, grain yield, and milling/baking quality; no variants were observed within the plot.

**2000:** F<sub>4:8</sub> State Advanced Yield Trial; WSU research land; selected based on appropriate plant height, maturity, field resistance to stripe rust, grain protein content, test weight, grain yield, and milling/baking quality; no variants were observed within the plot.

**2001:** F<sub>4:9</sub> State Advanced Yield Trial; WSU research land; selected based on appropriate plant height, maturity, field resistance to stripe rust, grain protein content, test weight, grain yield, and milling/baking quality; no variants were observed within the plot.

**2002:** F<sub>4:10</sub> WSU Commercial Variety Trial; Tri-State Variety Trial (WA, OR, ID), (tested as WA007921); selected based on appropriate plant height, head type, maturity, field resistance to stripe rust, grain protein content, test weight, grain yield, and milling/baking quality; no variants were observed within the plot.

**2003:** F<sub>4:11</sub> WSU Commercial Variety Trial; Tri-State Variety Trial (WA, OR, ID), Nursery, Western Regional Performance Nursery; selected based on appropriate plant height, head type, maturity, field resistance to stripe rust, grain protein content, test weight, grain yield, and milling/baking quality; no variants were observed within the plot.

Individual F<sub>4:11</sub> heads (1100) of Louise were hand threshed and separately planted in 10 ft rows (headrows) in March 2003 with irrigation in Othello, WA for Breeder seed production. Breeder seed was bulk harvested from a reselection of the headrow block, based on phenotypic uniformity, in August 2003 and planted in March 2004 for Foundation seed production; no variants were observed within the block.

**2004:** WA007921 approved for release as the cultivar 'Louise': PI 634865

**3. Evidence of uniformity and stability:**

Louise has been observed to be stable and uniform with respect to plant morphology since 1997 as an F<sub>4</sub>-derived line. This represents seven generations (1997-2003) through which this stability and uniformity have been observed. No variants were observed during this time frame.

**4. Variants during reproduction:**

Based on evaluations of Pre-Breeder, Breeder and Foundation seed lots, no variants were observed in Louise.

**EXHIBIT B. - STATEMENT OF DISTINCTNESS**

Louise was released as a replacement for the soft white spring variety 'Zak' in the intermediate to high rainfall (>400 mm of average annual precipitation), nonirrigated wheat production regions of Washington State based on its superior end-use quality, high grain yield potential, high-temperature adult-plant resistance to local races of stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici*), and partial resistance to the Hessian fly [*Mayetiola destructor* (Say)]. Louise is most similar to the soft white common varieties Wakanz and Wawawai.

**A. Agronomic Characteristics**

Heading date (day of year) and plant height (cm) data for Louise, Wakanz and Wawawai are described below. Number of plants used: 3 replications of 10 plants from each trial (150 plants for each variety overall).

1. Analysis of variance combined over locations indicates that the heading date of Louise is earlier than Wakanz (Mean = 1.5 days) and later than Wawawai (mean = 1.6 days) with an LSD of 0.4, ( $P \leq 0.1$ ) under Washington State field conditions. Data for each location, as well as over locations, are shown in Table B1.
2. Analysis of variance combined over locations indicates that Louise is taller than Wakanz (Mean = 9 cm), and shorter than Wawawai (Mean = 4 cm), with an LSD of 1.7, ( $P \leq 0.1$ ) under Washington State field conditions. Data for each location, as well as over locations, are shown in Table B1.

**Table B1.** Heading dates (Day of Year (DOY)) and plant heights (cm) of Louise, Wakanz and Wawawai in commercial variety trials at 5 locations in Washington State in crop year 2004.

crop year 2001

		Location					
Planting/Harvest Dates (DOY)		78/202	82/222	92/228	109/231	91/224	
Trait	Variety	Horse Heaven	Dusty	Reardan	Mayview	Almira	Mean
Heading Date (DOY)	Louise	152	164	176	179	170	165.1
	Wakanz	155	165	176	180	171	166.6
	Wawawai	150	163	174	176	170	163.5
	Mean	152.3	163.9	175.3	178.2	170.3	165.1
	LSD (10%)	1.0	0.6	1.0	1.2	1.4	0.4
	SD	2.0	1.1	1.0	1.7	1.0	8.7
	CV	0.4	0.2	0.3	0.4	0.5	0.4
Plant Height (cm)	Louise	73.7	94.8	92.3	76.2	78.7	85.1
	Wakanz	65.2	83.8	82.1	70.3	69.4	76.1
	Wawawai	77.9	100.8	94.0	83.8	83.8	89.0
	Mean	72.3	93.1	89.5	76.8	77.3	83.4
	LSD (10%)	1.5	5.7	4.7	4.3	6.2	1.7
	SD	5.7	7.8	5.9	6.6	7.0	14.9
	CV	1.2	3.5	3.0	3.2	4.6	3.3

\*Combined analysis of variance was conducted after the assumptions required for combining data over locations were met (i.e. variances were homogenous and data were normally distributed).

## B. Genetic Characteristics

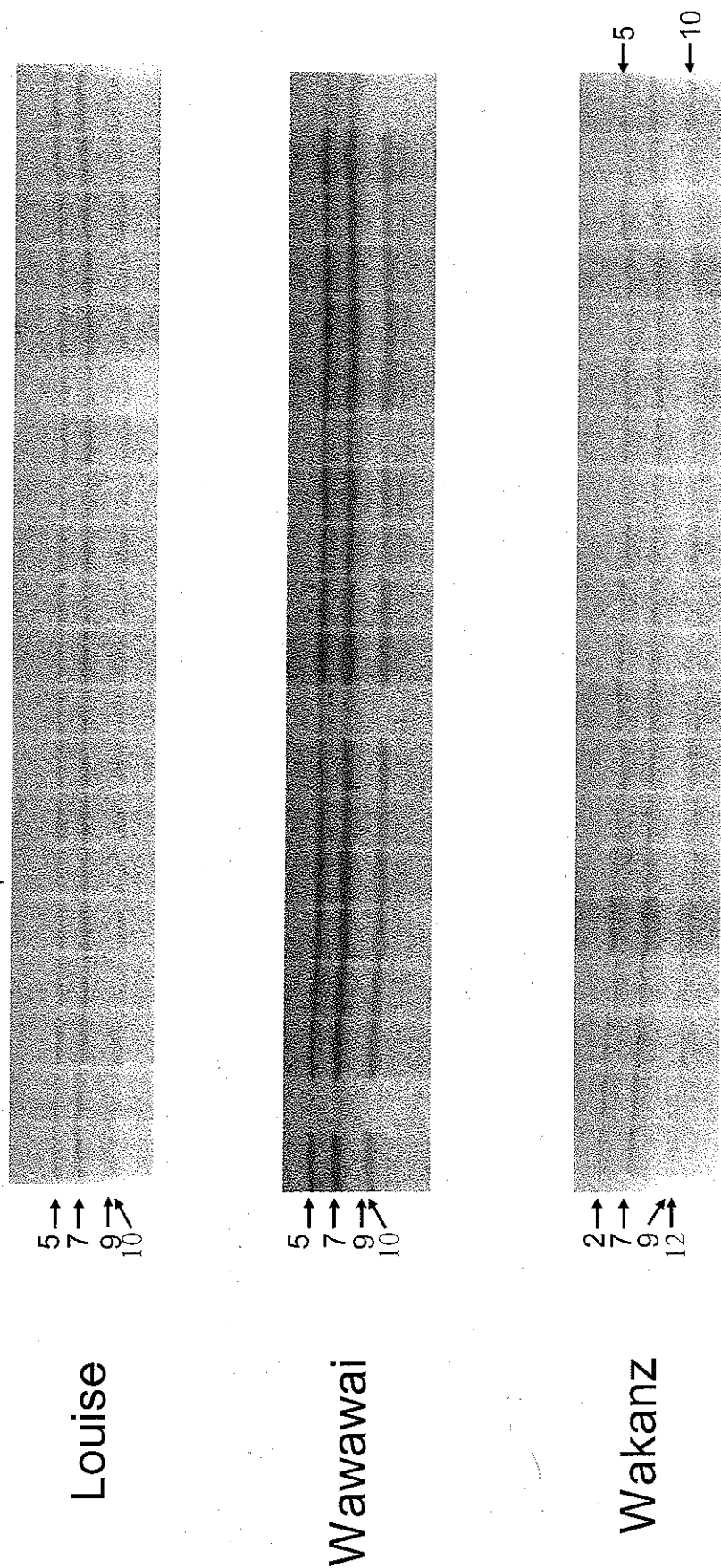
The novelty of Louise is demonstrated by high-molecular weight (HMW) glutenin profiles (Payne et al. 1983) and microsatellite fingerprint data that, when combined, differentiate Louise from Wawawai and Wakanz. HMW glutenins were resolved via SDS-Page, and visualized using Coomassie Brilliant Blue (Payne and Lawrence, 1983). Glutenin profiles were recorded using a Polaroid camera to capture gel images, which were scanned into the computer followed by labeling in Microsoft Powerpoint (Figure 1). Amplified fragments from microsatellite markers were resolved in denaturing polyacrylamide gels and visualized using a Li-cor DNA Sequencer (Li-cor, Lincoln, NE) (Figures 2 and 3).

The HMW glutenin profile of 100% of the individuals tested within Louise and Wawawai populations was [null 7+9 5+10] (Figure 1). The HMW glutenin profile of Wakanz was heterogeneous, consisting of the banding profiles [null 7+9 5+10] or [null 7+9 2+12] present in 35% and 65%, respectively, of individuals within the population.

Two microsatellite markers, *Xgwm132* and *Xgwm169*, were used to distinguish Louise from Wawawai and Wakanz. *Xgwm132* is located on wheat chromosome 6BS between RFLP loci *Xrz995* (proximal) and *Xcdo476* (distal), whereas *Xgwm16* is located on chromosome 6AL between the microsatellite locus *Xgwm570* (proximal) and RFLP locus *Xfba20* (distal) (Röder et al., 1998). DNA amplification of *Xgwm132* revealed 108 and 128 basepair (bp) fragments in Louise and Wawawai, whereas fragments of 106, 114 and 128 basepairs were amplified from Wakanz (Figure 2). DNA amplification of *Xgwm169* revealed a 192 bp fragment in Louise and Wakanz, and a 218 bp fragment in Wawawai (Figure 3).

## References:

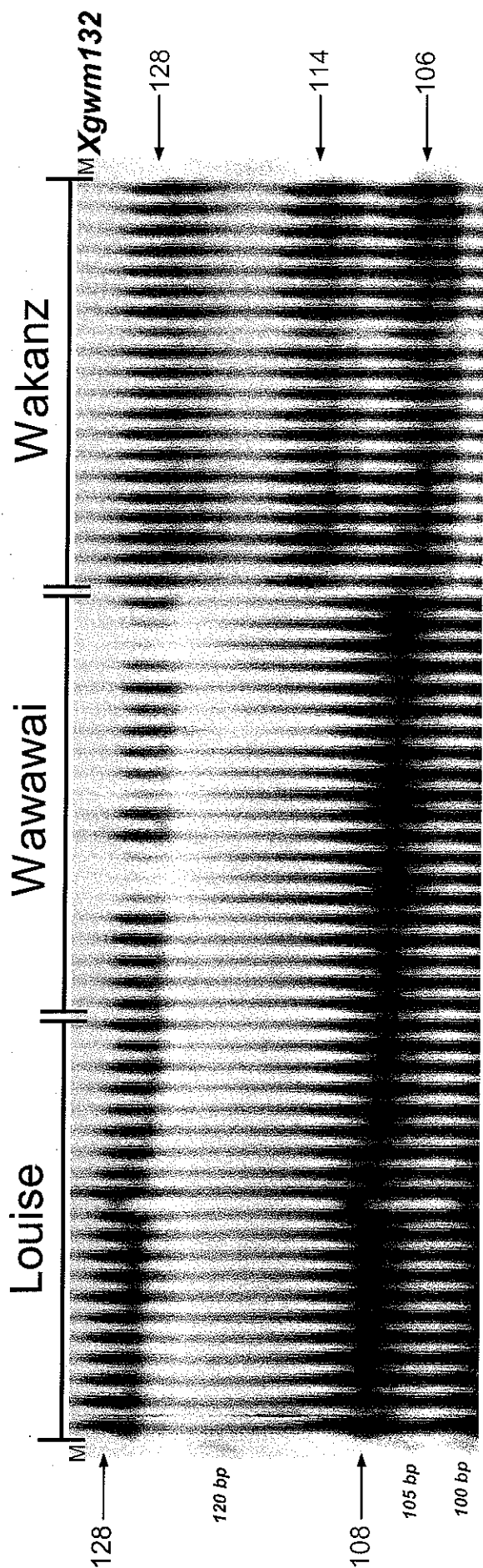
- Payne, PI, and GJ Lawrence. 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1, which code for high-molecular-weight subunits of glutenin in hexaploid wheat. *Cer. Res. Comm.* 11:29-35.
- Röder MS, V Korzun, K Wendehake, J Plaschke, M-H Tixier, P Leroy, and MW Ganal. 1998. A microsatellite map of wheat. *Genetics* 149:2007-2023.



**Figure 1.** High-molecular weight glutenin profiles of bulked (first lane) and individual (19) progeny extracts of Louise, Wawawai and Wakanz kernels. The HMW glutenin profile for Louise and Wawawai is [null 7+9 5+10], whereas individuals within Wakanz are either [null 7+9 2+12] or [null 7+9 5+10]. Proteins were resolved via SDS-Page, and visualized using Coomassie Brilliant Blue (Payne and Lawrence, 1983).

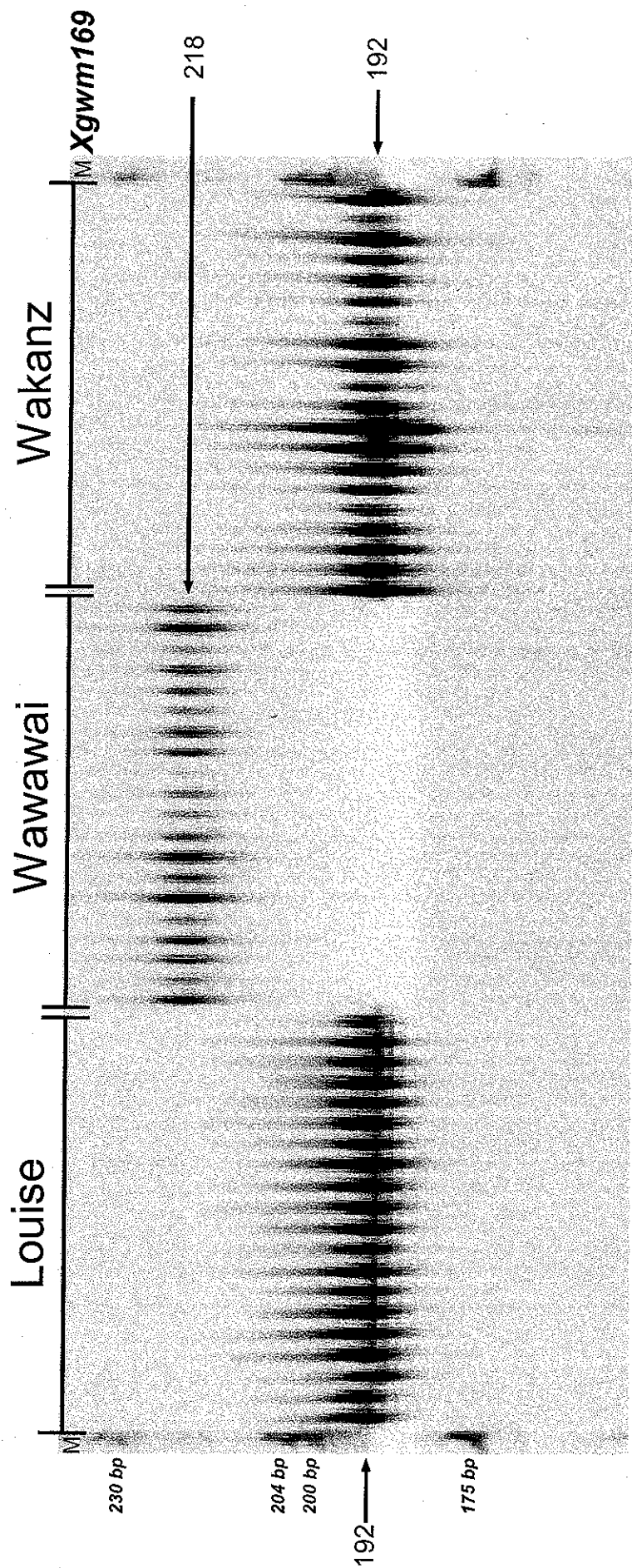
2005003 1.1





**Figure 2.** Microsatellite locus *Xgwm132* on wheat chromosome 6BS amplified from bulk (first lane of each variety) and individual (19) progeny of Louise, Wawawai and Wakanz. Two DNA fragments of 108 and 128 basepairs (bp) were amplified from Louise and Wawawai, whereas fragments of 106, 114 and 128 bp were amplified from Wakanz. DNA was obtained from young leaf tissue and the amplified products were resolved in denaturing polyacrylamide followed by visualization with an automated DNA Li-cor Sequencer (Li-cor, Lincoln, NE). The forward primer for *Xgwm132* was labeled with fluorescent dye detected at 700 nanometers. The first and last lanes, labeled "M", contain molecular weight standards at 75, 94, 100, 105 and 120 bp.

200500311



**Figure 3.** Microsatellite locus *Xgwm169* on wheat chromosome 6AL amplified from bulk (first lane of each cultivar) and individual (19) progeny of Louise, Wawawai and Wakanz. A 192 basepair (bp) fragment was amplified in Louise and Wakanz, whereas a 218 bp fragment was amplified from Wawawai. DNA was obtained from young leaf tissue and the amplified products were resolved in denaturing polyacrylamide followed by visualization with an automated DNA Li-cor Sequencer (Li-cor, Lincoln, NE). The forward primer for *Xgwm169* was labeled with fluorescent dye detected at 700 nanometers. The first and last lanes, labeled "M", contain molecular weight standards of 145, 175, 200 and 204 bp.

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 2.5 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

U.S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL MARKETING SERVICE  
SCIENCE AND TECHNOLOGY  
PLANT VARIETY PROTECTION OFFICE  
BELTSVILLE, MD 20705

Exhibit C

OBJECTIVE DESCRIPTION OF VARIETY  
Wheat (*Triticum* spp.)

NAME OF APPLICANT (S) Washington State University Research Foundation	TEMPORARY OR EXPERIMENTAL DESIGNATION WA007921	VARIETY NAME Louise
ADDRESS (Street and No. or RD No., City, State, Zip Code and Country) 1610 NE Eastgate Blvd. Pullman, WA 99163		FOR OFFICIAL USE ONLY PVPO NUMBER 200500311

## PLEASE READ ALL INSTRUCTIONS CAREFULLY:

Place the appropriate number that describes the varietal character of this variety in the boxes below. Place a zero in the first box (e.g.,    or   ) when number is either 99 or less or 9 or less respectively. Data for quantitative plant characters should be based on a minimum of 100 plants. Comparative data should be determined from varieties entered in the same trial. Royal Horticultural Society or any recognized color standard may be used to determine plant colors; designate system used: \_\_\_\_\_ Please answer all questions for your variety; lack of response may delay progress of your application.

## 1. KIND:

- 1 = Common  
2 = Durum  
3 = Club  
4 = Other (Specify) \_\_\_\_\_

## 2. VERNALIZATION:

- 1 = Spring  
2 = Winter  
3 = Other (Specify) \_\_\_\_\_

## 3. COLEOPTILE ANTHOCYANIN:

- 1 = Absent 2 = Present

## 4. JUVENILE PLANT GROWTH:

- 1 = Prostrate 2 = Semi-erect 3 = Erect

## 5. PLANT COLOR: (boot stage)

- 1 = Yellow-Green  
2 = Green  
3 = Blue-Green

## 6. FLAG LEAF: (boot stage)

- 1 = Erect 2 = Recurved  
 1 = Not Twisted 2 = Twisted  
 1 = Wax Absent 2 = Wax Present

## 7. EAR EMERGENCE:

- Number of Days (Average)  
 Number of Days Earlier Than \* Wakanz (PI 506342)  
Same As \* Eden (PI 630983)  
 Number of Days Later Than \* Wawawai (PI 574538)  
\*Relative to a PVPO-Approved Commercial Variety Grown in the Same Trial

## 8. ANTHR COLOR:

- 1 = Yellow 2 = Purple

**9. PLANT HEIGHT:** (from soil to top of head, excluding awns)

085

cm (Average)

09

cm Taller Than

Wakanz

Same As

04

cm Shorter Than

Wawawai

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**10. STEM:****A. ANTHOCYANIN**

1

1 = Absent 2 = Present

**B. WAXY BLOOM**

1

1 = Absent 2 = Present

**C. HAIRINESS** (last internode of rachis)

2

1 = Absent 2 = Present

**D. INTERNODE**

1

1 = Hollow

2 = Semi-solid

3 = Solid

4

Number of Nodes

**E. PEDUNCLE**

1

1 = Erect

2 = Recurved

3 = Semi-erect

32

cm Length

**F. AURICLE**

1

Anthocyanin:

1 = Absent

2 = Present

1

Hair:

1 = Absent

2 = Present

**11. HEAD: (At Maturity)****A. DENSITY**

1

1 = Lax

2 = Middense (Laxidense)

3 = Dense

**B. SHAPE**

1

1 = Tapering

2 = Strap

3 = Clavate

4 = Other (Specify) \_\_\_\_\_

**C. CURVATURE**

2

1 = Erect

2 = Inclined

3 = Recurved

**D. AWNEDNESS**

4

1 = Awnless

2 = Apically Awnletted

3 = Awnletted

4 = Awned

**12. GLUMES: (At Maturity)****A. COLOR**

1

1 = White

2 = Tan

3 = Other (Specify) \_\_\_\_\_

**B. SHOULDER**

5

1 = Wanting

2 = Oblique

3 = Rounded

4 = Square

5 = Elevated

6 = Apiculate

7 = Other (Specify) \_\_\_\_\_

**E. BEAK WIDTH**

1

1 = Narrow

2 = Medium

3 = Wide

**F. GLUME LENGTH**

3

1 = Short (ca. 7mm)

2 = Medium (ca. 8mm)

3 = Long (ca. 9mm)

**C. SHOULDER WIDTH**

2

1 = Narrow

2 = Medium

3 = Wide

**G. WIDTH**

3

1 = Narrow (ca. 3mm)

2 = Medium (ca. 3.5mm)

3 = Long (ca. 4mm)

**D. BEAK**

3

1 = Obtuse

2 = Acute

3 = Acuminate

## 13. SEED:

## A. SHAPE

- ☐ 3 1 = Ovate  
2 = Oval  
3 = Elliptical

## B. CHEEK

- ☐ 1 1 = Rounded  
2 = Angular

## C. BRUSH

- ☐ 2 1 = Short  
2 = Medium  
3 = Long
- ☐ 1 = Not Collared  
2 = Collared

## D. CREASE

- ☐ 1 1 = Width 60% or less of Kernel  
2 = Width 80% or less of Kernel  
3 = Width Nearly as Wide as Kernel
- ☐ 2 1 = Depth 20% or less of Kernel  
2 = Depth 35% or less of Kernel  
3 = Depth 50% or less of Kernel

## E. COLOR

- ☐ 1 1 = White  
2 = Amber  
3 = Red  
4 = Other (Specify) \_\_\_\_\_

## F. TEXTURE

- ☐ 2 1 = Hard  
2 = Soft  
3 = Other (Specify) \_\_\_\_\_

## G. PHENOL REACTION (See Instructions)

- ☐ 5 1 = Ivory 4 = Dark Brown  
2 = Fawn 5 = Black  
3 = Light Brown

## H. SEED WEIGHT

- ☐ 44 g/1000 Seed (Whole number only)

## I. GERM SIZE

- ☐ 3 1 = Small  
2 = Midsize  
3 = Large

## 14. DISEASE: PLEASE INDICATE THE SPECIFIC RACE OR STRAIN TESTED

(0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

- |   |   |
|---|---|
| <input type="checkbox"/> 0 Stem Rust ( <i>Puccinia graminis</i> f. sp. <i>tritici</i> ) | <input type="checkbox"/> 0 Leaf Rust ( <i>Puccinia recondita</i> f. sp. <i>tritici</i> )                      |
| <input type="checkbox"/> 4 Stripe Rust ( <i>Puccinia striiformis</i> )                  | <input type="checkbox"/> 0 Loose Smut ( <i>Ustilago tritici</i> )   |
| <input type="checkbox"/> 0 Tan Spot ( <i>Pyrenophora tritici-repentis</i> )             | <input type="checkbox"/> 0 Flag Smut ( <i>Urocystis agropyri</i> )  |
| <input type="checkbox"/> 0 Halo Spot ( <i>Selenophoma donacis</i> )                     | <input type="checkbox"/> 0 Common Bunt ( <i>Tilletia tritici</i> or <i>T. laevis</i> )                        |
| <input type="checkbox"/> 0 <i>Septoria nodorum</i> (Glume Blotch)                       | <input type="checkbox"/> 0 Dwarf Bunt ( <i>Tilletia controversa</i> )   |
| <input type="checkbox"/> 0 <i>Septoria avenae</i> (Speckled Leaf Disease)               | <input type="checkbox"/> 0 Karnal Bunt ( <i>Tilletia indica</i> )   |
| <input type="checkbox"/> 0 <i>Septoria tritici</i> (Speckled Leaf Blotch)               | <input type="checkbox"/> 0 Powdery Mildew ( <i>Erysiphe graminis</i> f. sp. <i>tritici</i> )                  |
| <input type="checkbox"/> 0 Scab ( <i>Fusarium</i> spp.)                                 | <input type="checkbox"/> 0 "Snow Molds"   |
| <input type="checkbox"/> 0 "Black Point" (Kernel Smudge)                                | <input type="checkbox"/> 0 Common Root Rot ( <i>Fusarium</i> , <i>Cochliobolus</i> and <i>Bipolaris</i> spp.) |
| <input type="checkbox"/> 0 Barley Yellow Dwarf Virus (BYDV)                             | <input type="checkbox"/> 1 Rhizoctonia Root Rot ( <i>Rhizoctonia solani</i> )                                 |
| <input type="checkbox"/> 0 Soilborne Mosaic Virus (SBMV)                                | <input type="checkbox"/> 0 Black Chaff ( <i>Xanthomonas campestris</i> pv. <i>translucens</i> )               |
| <input type="checkbox"/> 0 Wheat Yellow (Spindle Streak) Mosaic Virus                   | <input type="checkbox"/> 0 Bacterial Leaf Blight ( <i>Pseudomonas syringae</i> pv. <i>syringae</i> )          |
| <input type="checkbox"/> 0 Wheat Streak Mosaic Virus (WSMV)                             | <input type="checkbox"/> Other (Specify) _____  |
| <input type="checkbox"/> Other (Specify) _____  | <input type="checkbox"/> Other (Specify) _____  |
| <input type="checkbox"/> Other (Specify) _____  | <input type="checkbox"/> Other (Specify) _____  |
| <input type="checkbox"/> Other (Specify) _____  | <input type="checkbox"/> Other (Specify) _____  |

## 15. INSECT: (0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

PLEASE SPECIFY BIOTYPE (where needed)

- |  |  |
|--|--|
| <input type="checkbox"/> 3 Hessian Fly ( <i>Mayetiola destructor</i> )   | <input type="checkbox"/> Other (Specify) _____ |
| <input type="checkbox"/> 0 Stem Sawfly ( <i>Cephus</i> spp.)             | <input type="checkbox"/> Other (Specify) _____ |
| <input type="checkbox"/> 1 Cereal Leaf Beetle ( <i>Oulema melanopa</i> ) | <input type="checkbox"/> Other (Specify) _____ |

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15. INSECT: (continued)    0 = Not Tested    1 = Susceptible    2 = Resistant    3 = Intermediate    4 = Tolerant

PLEASE SPECIFY BIOTYPE (Where Needed)

<input type="checkbox"/> 0	Russian Aphid ( <i>Diuraphis noxia</i> )	<input type="checkbox"/>	Other (Specify) _____
<input type="checkbox"/> 0	Greenbug ( <i>Schizaphis graminum</i> )	<input type="checkbox"/>	Other (Specify) _____
<input type="checkbox"/> 0	Aphids	<input type="checkbox"/>	Other (Specify) _____

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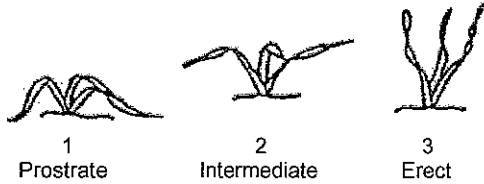
16. ADDITIONAL INFORMATION ON ANY ITEM ABOVE, OR GENERAL COMMENTS:

## WHEAT DESCRIPTOR ILLUSTRATIONS

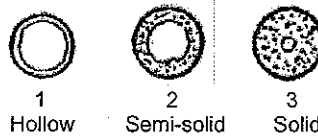
Section Numbers Correspond to the Numbers of the Sections on the Form

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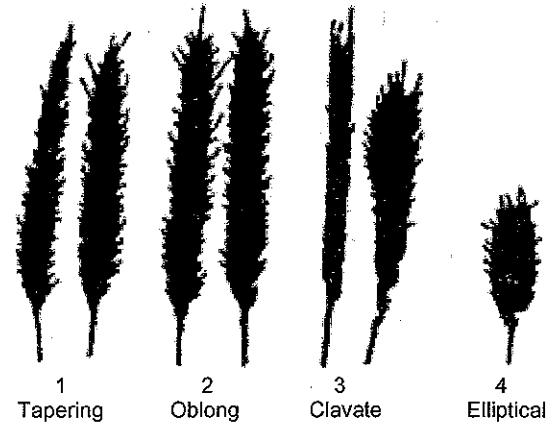
## 4. EARLY PLANT GROWTH HABIT:



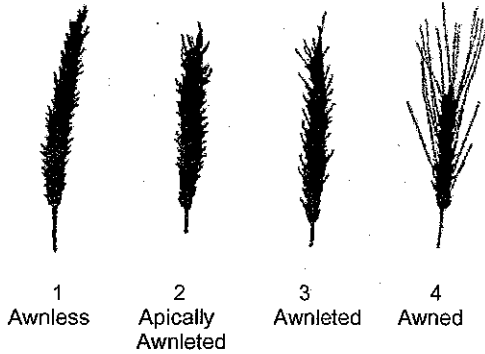
## 10. STEM INTERNODE X-SECTION:



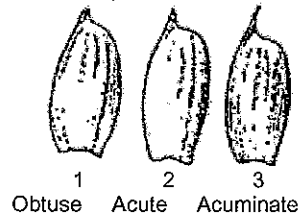
## 11. SPIKE SHAPE:



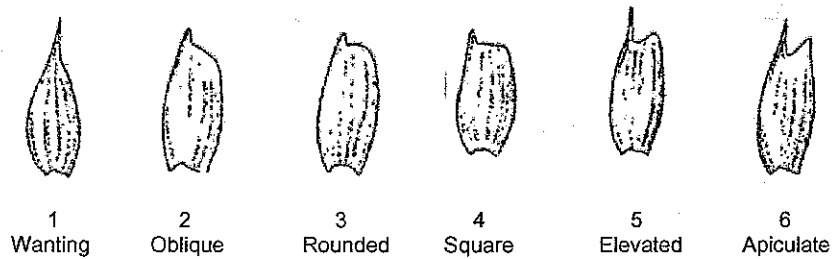
## 11. AWNEDNESS:



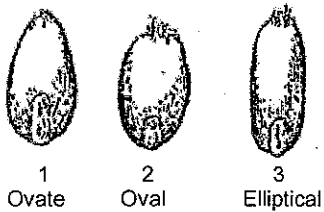
## 12. BEAK SHAPE:



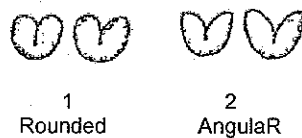
## 12. SHOULDER SHAPE:



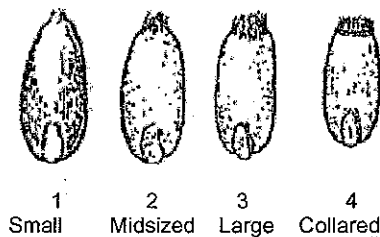
## 13. SEED SHAPE:



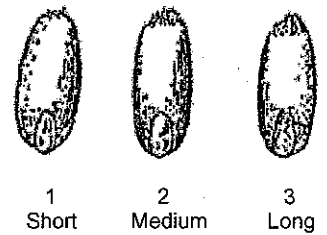
## 13. CHEEK SHAPE:



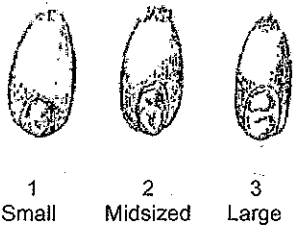
## 13. BRUSH SIZE



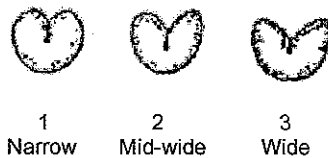
## 13. BRUSH HAIR LENGTH:



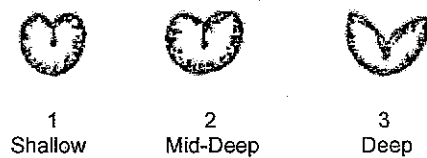
## 13. GERM (EMBRYO) SIZE:



## 13. SEED CREASE WIDTH:



## 13. SEED CREASE DEPTH:



**EXHIBIT D – OPTIONAL SUPPORTING INFORMATION****Milling and Baking Quality:**

The end-use quality performance of Louise is compared with the soft white spring varieties Alpowa and Zak through t-test analyses (Table D1). Alpowa is the primary soft white spring variety, based on acreage, in commercial production in Washington State, whereas Zak is the current regional standard for end-use quality of soft white spring wheat.

The grain test weight of Louise is similar to Zak, but significantly lower (poorer) than that of Alpowa. The grain protein concentration of Louise is similar to Alpowa, and lower (better) than Zak. The thousand kernel weight of Louise is heavier (better) than those for Alpowa and Zak. The flour yield of Louise is similar to Zak and higher (better) than Alpowa. The break flour yield of Louise is higher (better) than Alpowa, but lower (poorer) than Zak. The flour ash content of Louise is similar to that of Alpowa and lower (better) than that of Zak. The milling score of Louise is higher (better) than those for Alpowa and Zak. The flour protein concentrations of Louise, Alpowa and Zak are similar, as are the mixograph water absorption rates of flour extracted from the three varieties. Louise and Zak have similar cookie diameters, which are larger (better) than that for Alpowa. Sponge cake volumes of Louise, Alpowa and Zak are similar.

In general, Louise has excellent milling and baking properties. Of particular note is its high milling score and superior cookie diameter. Louise's milling performance is substantially better than that of Alpowa, and its baking qualities are similar to or better than those of Zak.



**Table D1:** Mean, least significant difference (LSD), probability level (P-value) and number of pair wise comparisons made (N) in t-test analyses for various end-use quality characteristics.

Variety	Test Weight (lb/bu)	Grain Protein (%)	Thousand Kernel Weight (g)	Flour Yield (%)	Break Flour Yield (%)	Flour Ash (%)	Milling Score	Flour Protein (%)	Mixograph Water Absorption (%)	Cookie Diameter (cm)	Sponge Cake Volume (cc)
Louise	61.1	10.7	37.6*	66.4*	49.3*	0.36	82.8*	8.8	53.2	9.7*	1327
Alpowa	62.5*	11.0	34.1	64.0	48.0	0.35	80.6	9.1	53.5	9.3	1368
LSD	0.6	0.5	2.1	0.9	0.9	0.02	1.7	0.4	0.7	0.2	148
P-value	<0.01	0.17	<0.01	<0.01	0.01	0.13	0.01	0.19	0.36	<0.01	0.35
N	15	15	14	15	15	15	15	15	13	13	3
Louise	61.6	10.7*	39.3*	67.1	49.9	0.36*	83.9*	8.9	53.0	9.7	1305
Zak	61.8	11.1	35.4	66.7	51.1*	0.39	81.2	9.2	53.1	9.7	1323
LSD	0.4	0.3	1.0	0.4	0.6	0.01	0.8	0.3	0.5	0.1	35
P-value	0.59	0.02	<0.01	0.06	<0.01	<0.01	<0.01	0.07	0.73	0.05	0.29
N	25	25	25	24	24	25	24	25	22	23	12

\*Significantly different ( $P \leq 0.05$ )

U.S. DEPARTMENT OF AGRICULTURE  
AGRICULTURAL MARKETING SERVICE**EXHIBIT E**  
**STATEMENT OF THE BASIS OF OWNERSHIP**

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). The information is held confidential until the certificate is issued (7 U.S.C. 2426).

1. NAME OF APPLICANT(S) Washington State University Research Foundation	2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER WA007921	3. VARIETY NAME Louise
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP, and Country) 1610 NE Eastgate Blvd. Pullman, WA 99163	5. TELEPHONE (Include area code) (509) 335-4363	6. FAX (Include area code) (509) 335-7237
7. PVPO NUMBER		200500311

8. Does the applicant own all rights to the variety? Mark an "X" in the appropriate block. If no, please explain. ☒ YES ☐ NO9. Is the applicant (individual or company) a U.S. national or a U.S. based company? If no, give name of country. ☒ YES ☐ NO10. Is the applicant the original owner? ☐ YES ☒ NO If no, please answer one of the following:

a. If the original rights to variety were owned by individual(s), is (are) the original owner(s) a U.S. National(s)?

☒ YES ☐ NO If no, give name of country

b. If the original rights to variety were owned by a company(ies), is (are) the original owner(s) a U.S. based company?

☐ YES ☐ NO If no, give name of country

11. Additional explanation on ownership (Trace ownership from original breeder to current owner. Use the reverse for extra space if needed):

'Louise' was developed by Dr. Kimberlee K. Kidwell, Spring Wheat breeder and geneticist at Washington State University.

Washington State University's ownership interests were assigned to the Washington State University Research Foundation.

**PLEASE NOTE:**

Plant variety protection can only be afforded to the owners (not licensees) who meet the following criteria:

1. If the rights to the variety are owned by the original breeder, that person must be a U.S. national, national of a UPOV member country, or national of a country which affords similar protection to nationals of the U.S. for the same genus and species.
2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by nationals of a country which affords similar protection to nationals of the U.S. for the same genus and species.
3. If the applicant is an owner who is not the original owner, both the original owner and the applicant must meet one of the above criteria.

The original breeder/owner may be the individual or company who directed the final breeding. See Section 41(a)(2) of the Plant Variety Protection Act for definitions.

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 0.1 hour per response, including the time for reviewing the instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, D.C. 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.